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ORIGINAL ARTICLE

Antimicrobial, spectral and thermal studies of divalent cobalt, nickel, copper and zinc complexes with triazole Schiff bases

Kiran Singh [a,*], Yogender Kumar [a], Parvesh Puri [a], Chetan Sharma [b], Kamal Rai Aneja [b]

[a] *Department of Chemistry, Kurukshetra University, Kurukshetra 136 119, India*
[b] *Department of Microbiology, Kurukshetra University, Kurukshetra 136 119, India*

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KEYWORDS

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Abstract Co(II), Ni(II), Cu(II) and Zn(II) complexes of bidentate Schiff bases derived from the condensation of 4-amino-5-mercapto-3-methyl/ethyl-1,2,4-triazole with 5-nitrofurfuraldehyde were synthesized and tested as antimicrobial agents. The Schiff bases and their metal complexes were characterized by elemental analyses, magnetic moment measurements, spectroscopic (IR, Electronic, ^{1}H NMR, ESR) and thermogravimetric analyses. A square planar geometry for Cu(II) and octahedral geometry for Co(II), Ni(II) and Zn(II) complexes have been proposed. The presence of coordinated water in metal complexes was confirmed by thermal and IR data of the complexes. The Schiff bases and their metal complexes have been screened for antibacterial [*Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*] and antifungal activities [*Aspergillus niger*, *A. flavus*].

1. Introduction

In view of the constant emergence of antibiotics' resistance to microorganisms, it is of critical importance to develop novel antibiotic classes, which eventually would target the lipoid layer of the organisms and other aspects of pathogen life cycle. Metal complexes may constitute one such possible class exhibiting biological activities (Chohan and Supran, 2005; Singh et al., 2010; Altundas et al., 2010). Schiff bases and their structural analogues, as ligating compounds containing acyclic and cyclic imines, are of great importance in modern coordination chemistry (Garnovskii et al., 2009) as they easily form stable complexes with most transition metal ions (Heshmatpour et al., 2007; Nuria et al., 2005). The five membered rings containing two or three nitrogen atoms (azoles) are more important, since the imidazole group of histidine acts as a ligand in most of the known hemoproteins. Imidazole derivatives and 1,2,4-triazoles (Hitchcock, 1991) are considered to be of potential interest to coordination chemists because they can be used to link studies on the metal binding properties of

* Corresponding author. Tel.: +91 9812137734.
E-mail address: kiransinghkuk@yahoo.co.in (K. Singh).
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nitrogenous bases with information on the proton affinities in the gas phase (Taft et al., 1986), in aqueous solutions and aprotic solvents (Catalan and Elguero, 1983), and on their H-bonding capacities (Ibraham et al., 1989). The coordination compounds of 1, 2, 4-triazoles are associated with diverse biological activities such as antimicrobial, anticonvulsant, fungicidal, antitumor, antiviral and analgesic activities (Kamble et al., 2010; Cui et al., 2009; Almasirad et al., 2010; Singh et al., 2006a,b).

Synthesis of new Schiff bases and their metal complexes are still the aim of many recent investigations (Lacroxi et al., 2004; Golcu et al., 2005; Omer et al., 2006), which prompted us to continue our previous studies on the characterization of transition metal complexes (Singh et al. 2006a,b). The present investigation deals with the synthesis, characterization, thermal and antimicrobial studies of Co(II), Ni(II), Cu(II) and Zn(II) complexes of Schiff bases derived from 4-amino-5-mercapto-3-methyl-1,2,4-triazole (ammt)/4-amino-3-ethyl-5-mercapto-1,2,4-triazole(aemt) and 5-nitrofurfuraldehyde. The synthesized Schiff bases and their metal complexes are new and to the best of our knowledge, these are not published elsewhere.

2. Experimental

2.1. Materials and physical measurements

All the chemicals used were of analytical grade. The metal contents were determined using standard gravimetric methods; cobalt was estimated as cobalt pyridine thiocyanate, nickel as nickel dimethylglyoximate, copper as cuprous thiocyanate and zinc as zinc ammonium phosphate (Vogel, 1999). Melting points were determined in open capillaries in electrical melting point apparatus, Perfit and are uncorrected. Carbon, hydrogen and nitrogen were determined using Perkin-Elmer 2400 Elemental Analyser at the Punjab University, Chandigarh. Electronic spectra of the metal complexes were recorded in DMF on a Systronics 2203 double beam spectrophotometer in the region of 1100–200 nm. IR spectra were recorded on a MB-3000 ABB Spectrometer in KBr/Nujol mulls in the range of 4000–250 cm^{-1}. Proton NMR spectra were recorded in DMSO-d_6 on a Bruker ACF 300 spectrophotometer at 300 MH_Z using tetramethyl silane as the internal standard. Magnetic moments were measured at IIC, IIT Roorkee on a vibrating sample magnetometer (Model 155). Thermal analysis of metal complexes was carried out in atmospheric air at the heating rate of 10°C min^{-1} using a Perkin Elmer (Pyris Diamond) instrument with reference to alumina powder.

2.2. Syntheses

4-Amino-5-mercapto-3-methyl-1,2,4-triazole (ammt) and 4-amino-3-ethyl-5-mercapto-1,2,4-triazole (aemt) were prepared by the reported literature method (Bala et al., 1978).

2.2.1. 4-(5-Nitrofurfuralideamino)-5-mercapto-3-methyl-1,2,4-triazole (HL^1)

A solution of *ammt* (2.00 g, 15 mmol) in ethanol (40 mL) was treated with 5-nitro-furfuraldehyde (2.17 g, 15 mmol). The reaction mixture was refluxed for 5 h and the solid crude was filtered off and washed with cold ethanol, and dried on a water bath and recrystallized from the same solvent. m.p. 181–183°C; Anal. for $C_8H_7N_5O_3S$ Calcd. C37.94, H2.79, N27.66. Found C37.33, H2.78, N27.61.

2.2.2. 4-(5-Nitrofurfuralideamino)-3-ethyl-5-mercapto-1,2,4-triazole (HL^2)

An ethanolic solution of *aemt* (2.00 g, 14 mmol) was added with stirring to an ethanolic solution of 5-nitro-furfuraldehyde (1.95 g, 14 mmol) and refluxed for 5 h and the product was filtered off, washed with ice cold ethanol and a wine red crystalline product was obtained after recrystallization from ethanol.

m.p. 151–154 °C; Anal. for $C_9H_9N_5O_3S$ Calcd. C40.45, H3.39, N26.20. Found C40.22, H3.30, N26.01.

2.2.3. Metal complexes of HL^1 (1:1)

The hot ethanolic (40–50°C) solutions of the HL^1 (0.26 g, 1 mmol) were added to the aqueous ethanolic (95%) solutions of metal acetates of Co(II) (0.26 g, 1 mmol), Ni(II) (0.25 g, 1 mmol), Cu(II) (0.19 g, 1 mmol) and Zn(II) (0.21 g, 1 mmol) respectively, which resulted in the immediate precipitation of metal derivatives. The products formed were filtered, washed with warm water, ethanol and finally with acetone and dried.

$Co(L^1)OAc.3H_2O$. Anal. for $C_{10}H_{15}N_5O_8SCo$ Calcd. C28.31, H3.56, N16.51, Co13.89. Found C28.11, H3.52, N16.49, Co13.82.

$Ni(L^1)OAc.3H_2O$. Anal. for $C_{10}H_{15}N_5O_8SNi$ Calcd. C28.33, H3.57, N16.52, Ni13.84. Found C28.29, H3.56, N16.50, Ni14.12.

$Cu(L^1)OAc.H_2O$. Anal. for $C_{10}H_{11}N_5O_6SCu$ Calcd. C30.57, H2.82, N17.83, Cu16.18. Found C30.56, H2.82, N17.81, Cu16.15.

$Zn(L^1)OAc.3H_2O$. Anal. for $C_{10}H_{15}N_5O_8SZn$ Calcd. C27.89, H3.51, N16.26, Zn15.18. Found C27.88, H3.48, N16.24, Zn15.10.

2.2.4. Metal complexes of HL^1 (1:2)

The aqueous ethanolic solutions (95%) of metal acetates of Co(II) (0.23 g, 0.9 mmol), Ni(II) (0.23 g, 0.9 mmol), Cu(II) (0.18 g, 0.9 mmol) and Zn(II) (0.20 g, 0.9 mmol) were treated respectively, with the hot ethanolic solutions (40–50°C) of the HL^1 (0.46 g, 1.8 mmol). The solid complexes, were filtered, washed with warm water, ethanol and finally with acetone and dried.

$Co(L^1)_2.2H_2O$. Anal. for $C_{16}H_{16}N_{10}O_8S_2Co$ Calcd. C32.06, H2.69, N23.37, Co9.83. Found C32.00, H2.67, N23.11, Co9.80.

$Ni(L^1)_2.2H_2O$. Anal. for $C_{16}H_{16}N_{10}O_8S_2Ni$ Calcd. C32.07, H2.69, N23.38, Ni9.80. Found C32.00, H2.62, N23.31, Ni9.74.

$Cu(L^1)_2$. Anal. for $C_{16}H_{12}N_{10}O_6S_2Cu$ Calcd. C33.83, H2.13, N24.66, Cu11.19. Found C33.31, H2.11, N24.61, Cu11.16.

$Zn(L^1)_2.2H_2O$. Anal. for $C_{16}H_{16}N_{10}O_8S_2Zn$ Calcd. C31.72, H2.66, N23.12, Zn10.79. Found C31.63, H2.59, N23.08, Zn10.68.

2.2.5. Metal complexes of HL^2 (1:1)

The hot ethanolic (40–50 °C) solutions of the HL^2 (0.26 g, 1 mmol) were added to the aqueous ethanolic solutions (95%) of metal acetates of Co(II) (0.25 g, 1 mmol), Ni(II) (0.25 g, 1 mmol), Cu(II) (0.20 g, 1 mmol) and Zn(II) (0.22 g, 1 mmol), respectively which resulted in the immediate precipitation of

metal derivatives. The products formed were filtered, washed with warm water, ethanol and finally with acetone and dried on water bath.

$Co(L^2)OAc.3H_2O$. Anal. for $C_{11}H_{17}N_5O_8SCo$ Calcd. C30.14, H3.91, N15.98, Co13.45. Found C30.04, H3.85, N15.90, Co13.30.

$Ni(L^2)OAc.3H_2O$. Anal. for $C_{11}H_{17}N_5O_8SNi$ Calcd. C30.16, H3.91, N15.99, Ni13.40. Found C30.00, H3.85, N15.92, Ni13.28.

$Cu(L^2)OAc.H_2O$. Anal. for $C_{11}H_{13}N_5O_6SCu$ Calcd. C32.47, H3.22, N17.21, Cu15.62. Found C32.44, H3.18, N17.20, Cu15.53.

$Zn(L^2)OAc.3H_2O$. Anal. for $C_{11}H_{17}N_5O_8SZn$ Calcd C29.71, H3.85, N15.75, Zn14.70. Found C29.65, H3.48, N15.73, Zn14.63.

2.2.6. Metal complexes of HL^2 (1:2)

The aqueous ethanolic solutions (95%) of metal acetates of Co(II) (0.21 g, 0.8 mmol), Ni(II) (0.21 g, 0.8 mmol), Cu(II) (0.17 g, 0.8 mmol) and Zn(II) (0.18 g, 0.8 mmol) were treated respectively, with the hot ethanolic (40–50°C) solutions of the HL^2 (0.45 g, 1.6 mmol). The solid complexes were filtered, washed with warm water, ethanol and finally with acetone and dried on a water bath.

$Co(L^2)_2.2H_2O$. Anal. for $C_{18}H_{20}N_{10}O_8S_2Co$ Calcd. C34.45, H3.21, N22.32, Co9.39. Found C34.23, H3.18, N22.29, Co9.24.

$Ni(L^2)_2.2H_2O$. Anal. for $C_{18}H_{20}N_{10}O_8S_2Ni$ Calcd. C34.47, H3.21, N22.33, Ni9.36. Found C34.40, H3.19, N22.33, Ni9.35.

$Cu(L^2)_2$. Anal. for $C_{18}H_{16}N_{10}O_6S_2Cu$ Calcd. C36.27, H2.71, N23.50, Cu10.66. Found C36.21, H2.68, N23.50, Cu10.55.

$Zn(L^2)_2.2H_2O$. Anal. for $C_{18}H_{20}N_{10}O_8S_2Zn$ Calcd. C34.10, H3.18, N22.09, Zn10.31. Found C34.01, H3.14, N22.02, Zn10.00.

2.3. Antimicrobial study

2.3.1. Test microorganisms

Totally six microbial strains were selected on the basis of their clinical importance in causing diseases in human. Two Gram-positive bacteria (*Staphylococcus aureus* MTCC 96 and *Bacillus subtilis* MTCC 121); two Gram-negative bacteria (*Escherichia coli* MTCC 1652 and *Pseudomonas aeruginosa* MTCC 741) and two fungi, *Aspergillus niger* and *A. flavus*, the ear pathogens were isolated from the patients of Kurukshetra (Aneja et al., 2010). All the bacterial cultures were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh. The bacteria were sub cultured on Nutrient agar whereas the fungi on Sabouraud's dextrose agar.

2.3.2. In-vitro antibacterial activity

The antibacterial activities of newly synthesized compounds were evaluated by the agar well diffusion method. All the microbial cultures were adjusted to 0.5 McFarland standards, which are visually comparable to a microbial suspension of approximately 1.5×10^8 cfu/ml. Mueller Hinton agar medium (20 mL) was poured into each Petri plate and plates were swabbed with 100 µl inocula of the test microorganisms and kept for 15 min for adsorption. Using a sterile cork borer of 8 mm diameter, wells were bored into the seeded agar plates and these were loaded with a 100 µl solution of each compound in dimethylsulphoxide (DMSO) with a concentration of 4.0 mg/ml. All the plates were incubated at 37°C for 24 h. Antibacterial activity of each synthetic compound was evaluated by measuring the zone of growth inhibition against the test organisms with a zone reader (Hi Antibiotic zone scale). DMSO was used as a negative control whereas Ciprofloxacin was used as a positive control. This procedure was performed in three replicate plates for each organism (Ahmad and Beg, 2001; Andrews, 2001).

2.3.3. Minimum inhibitory concentration (MIC)

MIC of the various compounds against bacterial strains was tested through a macro dilution tube method as recommended by NCCLS. In this method, the various test concentrations of chemically synthesized compounds were made from 128 to 0.25 µg/ml in sterile tubes no. 1 to 10. Hundred microlitres of sterile Mueller Hinton Broth (MHB) was poured into each sterile tube followed by the addition of 200 µl of the test compound in tube 1. Two fold serial dilutions were carried out from tube 1 to tube 10 and excess broth (100 µl) was discarded from the last tube no. 10. To each tube, 100 µl of standard inoculums (1.5×10^8 cfu/ml) was added. Ciprofloxacin was used as control. Turbidity was observed after incubating the inoculated tubes at 37°C for 24 h (NCCLS, 2000).

2.3.4. In-vitro antifungal activity

The antifungal activities of Schiff bases and their metal complexes were evaluated by poison food technique. The moulds were grown on Sabouraud dextrose agar (SDA) at 25°C for 7 days and used as inoculates. 15 ml of molten SDA (45°C) was poisoned by the addition of 100 µl volume of each compound having a concentration of 4.0 mg/ml reconstituted in the DMSO, poured into a sterile Petri plate and allowed it to solidify at room temperature. The solidified poisoned agar plates were inoculated at the centre with fungal plugs (8 mm diameter) obtained from the colony margins and incubated at 25°C for 7 days. DMSO was used as the negative control whereas Fluconazole was used as the positive control. The experiments were performed in triplicates. Diameter of fungal colonies was measured and expressed as percent mycelial inhibition by applying the formula (Al-Burtamani et al., 2005).

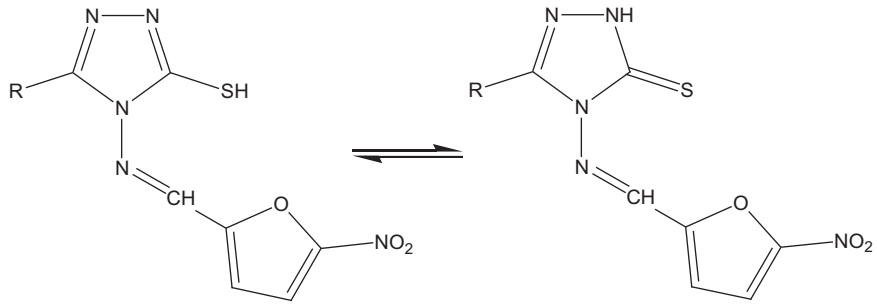
$$\text{Percent inhibition of mycelial growth} = (dc - dt)/dc \times 100$$

where dc is the average diameter of the fungal colony in negative control sets and dt is the average diameter fungal colony in the experimental sets.

3. Results and discussion

3.1. Chemistry

Analytical data indicated the formation of 1:1 and 1:2 metal complexes of HL^{1-2} with Co(II), Ni(II), Cu(II) and Zn(II) metal ions. The Schiff bases (Fig. 1) were soluble in ethanol and methanol. All the metal complexes are coloured, non hygroscopic solids, stable in air, infusible at higher temperature, insoluble in water and many common organic solvents but they are soluble in DMF and DMSO. The molar conductance values of the complexes (measured in 10^{-3} M DMF) are in the range of 1.5–3.6 $ohm^{-1}\,cm^2\,mol^{-1}$ indicating the



R = -CH_3, -CH_2CH_3

Figure 1 Structure of Schiff base.

non electrolytic nature (Singh et al., 2006). The purity of ligands and their metal complexes has been checked by TLC.

3.2. IR spectra

A band at 1605–1620 cm^{-1} in the spectra of Schiff bases had been assigned to the azomethine group frequency ν (N=CH), which shifted towards the lower frequency (Singh et al., 2006) by 15–20 cm^{-1} in the spectra of the complexes, indicating coordination through the nitrogen atom. Further coordination of metal to azomethine nitrogen was confirmed by a new band around 483–528 cm^{-1} due to ν (M–N). The Schiff bases exhibited a weak broad band around ~2700 cm^{-1} due to ν (S–H) vibrations (Singh et al., 2006; Kulkarni et al., 2010). This band disappeared in the spectra of the metal complexes indicating deprotonation and complexation through sulphur. In the spectra of metal complexes bands that appeared at 732–750 cm^{-1} and 334–348 cm^{-1} were assigned to ν (C–S) and ν (M–S), respectively. The presence of coordinated water molecules in the metal complexes was indicated by a broad trough band in the region of 3200–3450 cm^{-1}. A strong band in the region of 1722–1735 cm^{-1} had been assigned to ν ($OOCCH_3$) in 1:1 metal complexes (Table 1).

3.3. ^1H NMR Spectra

The ^1H NMR spectra have been recorded for Schiff bases (Fig. 2) and Zn(II) complexes (Table 2). In the NMR spectra of complexes, a shift of electron density from the ligand to metal had been observed. The ligands [HL^{1-2}] that showed singlets at δ 10.2 and 10.3 ppm, respectively were assigned to the azomethine proton. The signal due to the azomethine proton deshielded in the spectra of metal complexes, suggesting the coordination of metal ion to the azomethine nitrogen atom of the ligands (Singh et al., 2006; Bagihalli et al., 2009). In addition to this, the signal at δ 11.0–11.1 ppm was ascribed to the SH proton. Disappearance of SH protons signal in the spectra of metal complexes supported the deprotonation of the thiol group. In the spectra of Schiff bases two doublets at δ 7.1–7.2 and δ 7.4 ppm were assigned to the aromatic protons. These doublets show a slight shift upon coordination. In the spectra of 1:1 Zn complexes a singlet at δ 2.3 ppm was due to methyl group of the acetate ion.

3.4. Electronic spectra and magnetic measurements

The electronic spectra of Co(II) complexes (Table 3) exhibited absorption bands in the region of ~10800–11000 cm^{-1} and

Table 1 IR spectral data of the ligands and their metal complexes (cm^{-1}).

Compound	ν(N=CH)	ν(C–S)	ν(S–H)	ν($OOCCH_3$)	ν(H_2O/OH)	ν(M–S)	ν(M–N)
HL^1	1605	–	2669	–	–	–	–
$Co(L^1)(OAc)\ 3H_2O$	1593	741	–	1722	3294	343	528
$Co(L^1)_2\ 2H_2O$	1594	745	–	–	3288	335	521
$Ni(L^1)(OAc)\ 3H_2O$	1585	741	–	1728	3371	348	522
$Ni(L^1)_2\ 2H_2O$	1588	741	–	–	3294	355	520
$Cu(L^1)(OAc)\ H_2O$	1595	749	–	1733	3333	348	495
$Cu(L^1)_2$	1593	748	–	–	–	348	489
$Zn(L^1)(OAc)\ 3H_2O$	1588	750	–	1735	3441	342	480
$Zn(L^1)_2\ 2H_2O$	1592	741	–	–	3441	342	483
HL^2	1620	–	2675	–	–	–	–
$Co(L^2)(OAc)\ 3H_2O$	1608	730	–	1728	3311	334	510
$Co(L^2)_2.2H_2O$	1609	733	–	–	3310	336	512
$Ni(L^2)(OAc).3H_2O$	1607	735	–	1728	3298	345	495
$Ni(L^2)_2\ 2H_2O$	1618	733	–	–	3387	348	501
$Cu(L^2)(OAc)\ H_2O$	1612	732	–	1726	3410	345	528
$Cu(L^2)_2$	1612	733	–	–	–	348	525
$Zn(L^2)(OAc)\ 3H_2O$	1610	741	–	1730	3418	347	498
$Zn(L^2)_2\ 2H_2O$	1612	742	–	–	3418	345	498

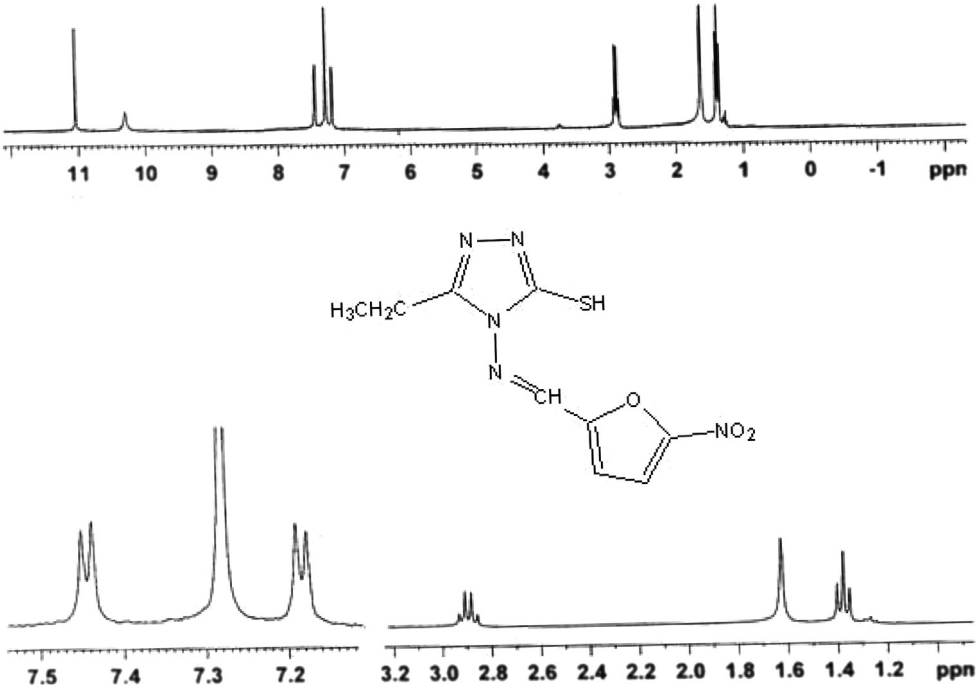


Figure 2 ^{1}H NMR spectra of 4-(5-nitrofurfuralideamino)-3-ethyl-5-mercapto-1,2,4-triazole.

Table 2 ^{1}H NMR spectral data of Schiff bases and their metal complexes.

Compounds	^{1}H NMR (DMSO-d_6) (ppm)
HL^1 [$C_8H_7N_5O_3S$]	2.5 (s, 3H, –CH_3), 7.1 (d, 1H, Ar–H), 7.4 (d, 1H, Ar H) 10.2 (s,1H, –N═CH–), 11.1 (s, 1H, –SH)
$Zn(L^1)OAc.3H_2O$ [$C_{10}H_{15}N_5O_8SZn$]	2.5(s, 3H, –CH_3), 7.6 (d, 1H, Ar–H), 7.8 (d, 1H, Ar–H) 10.4(s, 1H, –N═CH–), 2.3(s,3H, C**H$_3$**COO)
$Zn(L^1)_2.2H_2O$ [$C_{16}H_{16}N_{10}O_8S_2Zn$]	2.5(s, 6H, –CH_3), 7.6 (d, 2H, Ar–H), 7.9 (d, 2H, Ar–H) 10.5(s, 2H, –N═CH–)
HL^2 [$C_9H_9N_5O_3S$]	2.9 (q, 2H, –CH_2CH_3), 1.4 (t, 3H, –CH_2CH_3), 7.2 (d, 1H, Ar–H), 7.4 (d, 1H, Ar H) 10.3 (s,1H, –N═CH–), 11.0 (s, 1H, –SH)
$Zn(L^2)OAc.3H_2O$ [$C_{11}H_{17}N_5O_8SZn$]	2.7 (q, 2H, –CH_2CH_3), 1.3 (t, 3H, –CH_2CH_3), 7.6 (d, 1H, Ar–H), 7.8 (d, 1H, Ar H), 10.4 (s,1H, –N═CH–), 2.3(s,3H, C**H$_3$**COO)
$Zn(L^2)_2$ $2H_2O$ [$C_{18}H_{20}N_{10}O_8S_2Zn$]	2.8 (q, 4H, –CH_2CH_3), 1.3(t, 6H, –CH_2CH_3), 7.6 (d, 2H, Ar–H), 7.8 (d, 2H, Ar H) 10.4 (s,2H, –N═CH–)

Table 3 Electronic spectra and Ligand field parameters of metal complexes.

Compound	Transitions (cm^{-1})			Dq cm^{-1}	B cm^{-1}	v_2/v_1	β	β%
	v_1	v_2	v_3					
$Co(L^1)(OAc)$ $3H_2O$	10985	23206[a]	21002	1222.1	750.26	2.11	0.772	22.8
$Co(L^1)_2$ $2H_2O$	10988	23212[a]	20990	1222.4	749.06	2.11	0.771	22.9
$Ni(L^1)(OAc)$ $3H_2O$	9912	16523	24865	991.2	776.81	1.66	0.735	26.5
$Ni(L^1)_2$ $2H_2O$	9934	16458	24880	993.4	769.06	1.65	0.728	27.2
$Co(L^2)(OAc)$ $3H_2O$	10883	22937[a]	19998	1205.4	685.76	2.10	0.706	29.4
$Co(L^2)_2$ $2H_2O$	10892	22954[a]	19985	1206.2	672.22	2.10	0.692	30.8
$Ni(L^2)(OAc)$ $3H_2O$	9876	16220	24992	987.6	772.26	1.64	0.731	26.9
$Ni(L^2)_2$ $2H_2O$	9887	16315	24885	988.7	769.26	1.65	0.728	27.2

[a] Calculated.

~19800–21000 cm^{-1} corresponding to v_1 and v_3 transitions respectively, which are attributed to the transitions $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ (v_1); $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ (v_3) (Singh et al., 2006; Avaji et al., 2008). These bands are characteristic of high spin octahedral Co(II) complexes; v_2 was not observed, but it can be calculated (Cotton and Wilkinson, 1988; Patra et al., 2005) by using relation $v_2 = v_1 + 10$ Dq, which was very close to the v_3 transition. The ligand field parameter such as Dq, B^1, β and β% have been calculated by using Band-fitting equation given by Underhill and Billing (Lever, 1968). The crystal field

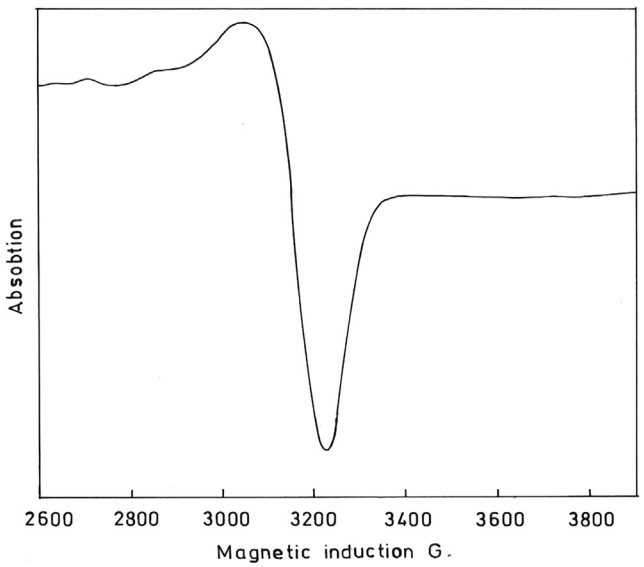


Figure 3 X-Band ESR spectra of $Cu(L^1)(OAc).H_2O$.

splitting energy (Dq) value was found to be $\sim$1200 cm^{-1}. These values were well within the range reported for the octahedral complexes (Lever, 1968). The Rachah parameter (B) was found to be 672–772 cm^{-1} which is less than the free ion value of 971 cm^{-1}, suggesting an orbital overlap and delocalization of the electron on the metal ion. The nephelauxetic ratio (β) for the 1:1 and 1:2 cobalt complexes is less than one suggesting partial covalency in the metal ligand bond. The values Dq, β%, v_2/v_1 (Table 3) suggest the distorted octahedral geometry for the Co(II) complexes.

The 1:1 and 1:2 Ni(II) complexes that exhibit three absorption bands in the regions of $\sim$9800–9950 cm^{-1}, $\sim$16200–16600 cm^{-1} and $\sim$24800–25000 cm^{-1} were assigned to $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ (v_1), $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ (v_2) and $3A_{2g}(F) \rightarrow 3T_{1g}(P)$ (v_3) transitions, respectively, indicated distorted octahedral geometry for the Ni(II) ion. By using the Band-fitting equation (Lever, 1968) ligand field parameters have been calculated for Ni(II) complexes. Rachah parameter B is less than the free ion value i.e. 1041 cm^{-1} indicating the covalent character of the metal ligand bond. The crystal field splitting energy (Dq) value was found to be $\sim$990 cm^{-1} suggesting octahedral geometry for Ni(II) complexes (Mohammed, 2001). The electronic spectra of Cu(II) complexes showed a broad band at $\sim$18500 cm^{-1}, which is attributed to $^2B_1g \rightarrow {}^2A_1g$ (v_1) transition. It is a characteristic band of square planar geometry around the Cu (II) (Osman et al., 2004).

The 1:1 and 1:2 Co(II) complexes show a magnetic moment (μ_{eff}) of 4.72–4.89 BM, well within the expected range of the octahedral complex (4.3–5.2 BM) (Singh et al., 2006; Avaji et al., 2008). The nickel complexes show a magnetic moment of 3.21–3.30 BM, suggested distorted octahedral geometry. The Cu(II) complex had magnetic moment value 1.72–1.75 BM, which falls in the normal range (1.7–2.2 BM).

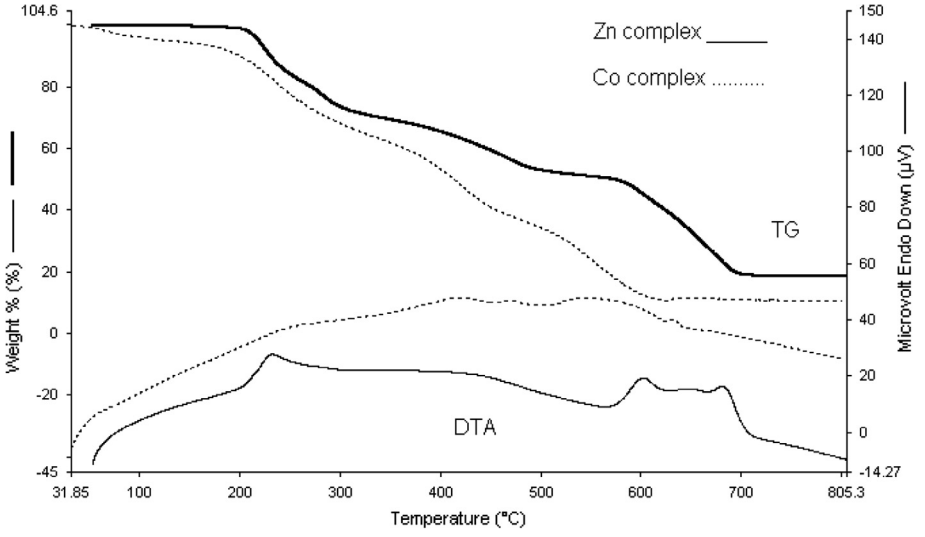


Figure 4 TG and DTA thermograms of $Co(L^1)_2.2H_2O$ and Zn $(L^2)OAc.3H_2O$.

Table 4 Themogravimetric characteristics of metal complexes.

Compound	Process	Temp, °C	Product	Mass,%		No. of Moles	Residue, %		Nature
				Calcd.	Found		Calcd	Found	
$Co(L^1)_2$ $2H_2O$	Dehydration	50–170	H_2O	6.01	6.80	2			
	Dec. of coordination	170–400	–Org. moiety	41.73	42.00	2			
	Sphere	400–650	–Triazole	42.40	41.88	2	12.50	11.47	CoO
$Zn(L^2)OAc.3H_2O$	Dehydration	50–235	H_2O	12.19	12.01	3			
	Dec. of coordination	235–593	–OAc, –Org. moiety	41.53	40.99	1,1			
	Sphere(L)	360–750	–Triazole	32.06	33.00	1	18.04	18.30	ZnO

3.5. ESR spectra

ESR spectra for 1:1 and 1:2 copper(II) complexes i.e. $Cu(L^1)(OAc).H_2O$ (Fig. 3) and $Cu(L^1)_2$ had been recorded. From the observed g values for $Cu(L^1)(OAc).H_2O$ at room temperature ($g_{||\ ||}$ = 2.20, $g_{\perp}$ = 2.070, g_{av} = 2.11, G = 2.89) and for $Cu(L^1)_2$ at room temperature ($g_{||\ ||}$ = 2.189, $g_{\perp}$ = 2.060, g_{av} = 2.103, G = 3.19), it is evident that unpaired electron is localized in the dx^2–y^2 orbital and corresponds to square planar geometry of the complexes. The $g_{||\ ||} < 2.3$ value confirms the covalent character of the metal ligand bond. The axial symmetry parameter G is less than 4.0, indicates considerable exchange interaction in the solid complex (Dutta and Syamal, 1993).

3.6. Thermal analysis

Thermo gravimetric studies were carried out for $Co(L^1)_2.2H_2O$ and $Zn(L^2)(OAc)\ 3H_2O$ (Fig. 4) (Singh et al., 2006). The decomposition temperature, pyrolysed products, percentage mass loss of the complexes and the ash (percent) are given in Table 4. The Co(II) complex decomposes in three stages. The first step, 50–170°C, results in a mass loss (found 6.80%, calcd. 6.01%), corresponding to a loss of two water molecules of hydration. The anhydrous complex decomposes in a major stage consisting of two overlapping steps. In the first stage, the organic part started decomposing, giving metal-triazole at 400°C with a mass loss of 42.00% on the TG curve (calcd. 41.73). In the temperature range of 400–650°C all the triazole parts get decomposed with a mass loss of 41.88% (Calcd. 42.40%). The Zn(II) complex also decomposes in three stages. The first step 50–235°C, results in a mass loss (found 12.01%, calcd. 12.19%) corresponding to a loss of three water molecules of hydration. In the second step, the organic part was lost in the 235–593°C range with a mass loss of 40.99% (calcd. 41.53%), giving the metal triazole. The third step corresponds to the decomposition of triazole molecules with mass loss of 33.00% (calcd. 32.06%) in the TG curve. The decomposition of both the complexes ended with oxide formation.

3.7. Antimicrobial activity

All the newly synthesized Schiff bases and their metal complexes were screened for their antibacterial and antifungal activities. All the tested chemical compounds possessed variable antibacterial activity against both Gram-positive (*S. aureus* and *B. subtilis*) and Gram negative (*E. coli* and *P. aeruginosa)* bacteria and antifungal activity against *A. niger* and *A. flavus*. Positive controls produced significant sized

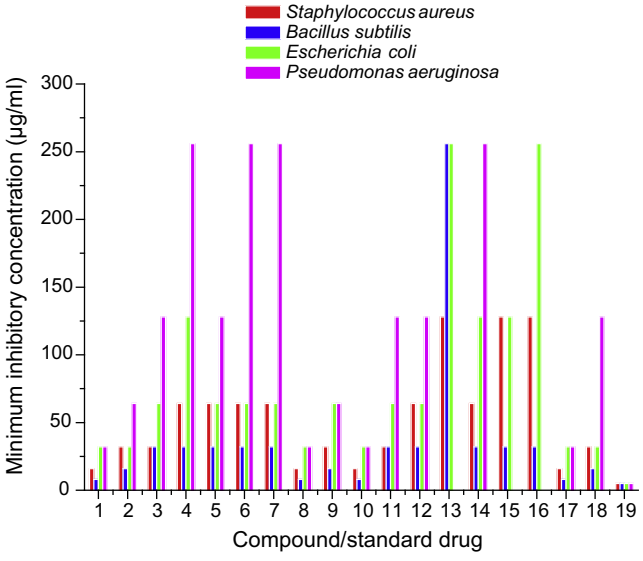


Figure 5 Minimum inhibitory concentrations (µg/ml) of compounds with standard drug.

Table 5 Antibacterial activity of synthesized compounds.

Sr. No.	Compounds	Diameter of growth of inhibition zone (mm)[a]			
		Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*
1	HL^1	24.3	26.6	22.3	18.3
2	$Co(L^1)(OAc)\ 3H_2O$	22.6	26.8	21.6	18.6
3	$Co(L^1)_2\ 2H_2O$	22.3	23.6	23.3	16.0
4	$Ni(L^1)(OAc)\ 3H_2O$	18.6	22.0	17.6	19.3
5	$Ni(L^1)_2\ 2H_2O$	19.0	21.6	18.6	15.6
6	$Cu(L^1)(OAc)\ H_2O$	20.6	23.6	19.3	14.6
7	$Cu(L^1)_2$	19.6	22.3	20.3	16.0
8	$Zn(L^1)(OAc)\ 3H_2O$	24.3	26.9	22.6	19.6
9	$Zn(L^1)_2\ 2H_2O$	23.6	25.6	20.3	18.6
10	HL^2	25.6	26.6	21.3	19.3
11	$Co(L^2)(OAc)\ 3H_2O$	21.0	23.6	20.6	16.3
12	$Co(L^2)_2\ 2H_2O$	20.6	22.3	19.0	15.6
13	$Ni(L^2)(OAc)\ 3H_2O$	16.3	14.6	13.6	–
14	$Ni(L^2)_2\ 2H_2O$	18.6	23.0	17.6	14.3
15	$Cu(L^2)(OAc)\ H_2O$	16.6	22.3	15.3	–
16	$Cu(L^2)_2$	15.3	22.6	14.0	–
17	$Zn(L^2)(OAc)\ 3H_2O$	24.6	26.3	22.3	19.6
18	$Zn(L^2)_2\ 2H_2O$	23.3	25.6	21.3	16.3
19	Ciprofloxacin	26.6	24.0	25.0	22.0

[a] Values, including diameter of the well (8 mm), are means of three replicates.

inhibition zones against the tested bacteria and fungi; however, negative control produced no observable inhibitory effect against any of the test organisms as shown in Table 5.

On the basis of maximum inhibitory activity shown against Gram positive bacteria, four compounds namely HL^1, $Zn(L^1)(OAc).3H_2O$, HL^2 and $Zn(L^2)(OAc).3H_2O$ were found to be most effective against *S. aureus* with a zone of inhibition ranging between 25 mm and 24 mm and with *B. subtilis* a zone of inhibition is 26 mm. However in case of Gram negative bacteria, three compounds, HL^1, $Zn(L^1)(OAc).3H_2O$ and $Zn(L^2)(OAc).3H_2O$ were found to be most effective *E. coli* with the zone of inhibition 22 mm and compounds $Zn(L^1)(OAc).3H_2O$, HL^2 and $Zn(L^2)(OAc).3H_2O$ against *P. aeruginosa* with a zone of inhibition of 19 mm, whereas, four compounds, $Ni(L^2)(OAc).3H_2O$, $Cu(L^2)(OAc).H_2O$ and $Cu(L^2)_2$ do not exhibit any activity against *P. aeruginosa* (Table 5). Gram negative bacteria showed low activity as compared to Gram positive bacteria against the tested chemical compounds.

Table 6 Antifungal activity of synthesized compounds.

Compounds	Mycelial growth inhibition level, %	
	Aspergillus niger	*Aspergillus flavus*
HL^1 (nfmmt)	66.6	63.3
$Co(L^1)(OAc)\ 3H_2O$	55.5	51.1
$Co(L^1)_2\ 2H_2O$	50	48.8
$Ni(L^1)(OAc)\ 3H_2O$	58.8	61.1
$Ni(L^1)_2\ 2H_2O$	52.2	48.8
$Cu(L^1)(OAc)\ H_2O$	48.8	51.1
$Cu(L^1)_2$	53.3	50
$Zn(L^1)(OAc)\ 3H_2O$	63.3	66.6
$Zn(L^1)_2\ 2H_2O$	58.8	61.1
HL^2 (nfemt)	68.8	63.3
$Co(L^2)(OAc)\ 3H_2O$	62.2	65.5
$Co(L^2)_2\ 2H_2O$	53.3	51.1
$Ni(L^2)(OAc)\ 3H_2O$	52.2	55.5
$Ni(L^2)_2\ 2H_2O$	58.8	62.2
$Cu(L^2)(OAc)\ H_2O$	51.1	53.3
$Cu(L^2)_2$	50	52.2
$Zn(L^2)(OAc)\ 3H_2O$	63.3	65.5
$Zn(L^2)_2\ 2H_2O$	61.1	62.2
Fluconazole	81.1	77.7

All the compounds, screened for their antifungal activity, showed more than 60% inhibition of mycelial growth against *A. niger* and *A. flavus*. $Zn(L^2)(OAc).3H_2O$ showed the highest inhibition of fungal mycelium (68%) against *A. niger* (Table 6). The antimicrobial studies suggested that all the Schiff bases were found to be biologically active and their metal complexes showed significantly enhanced antibacterial and antifungal activity against microbial strains in comparison to the free ligands. It was suggested (Chohan and Praveen, 2001; Chohan et al., 2004) that chelation tended to make the ligands act as more powerful and potent bactereostatic agents, thus inhibiting the growth of bacteria more than the parent ligands did. It was suspected that factors such as solubility, conductivity, dipole moment and cell permeability mechanism influenced by the presence of metal ion might be the possible reason for the increase in activity.

4. Conclusions

The synthesized Schiff bases (HL^{1-2}) act as bidentate ligands and coordinated to the metal ion through nitrogen and sulphur

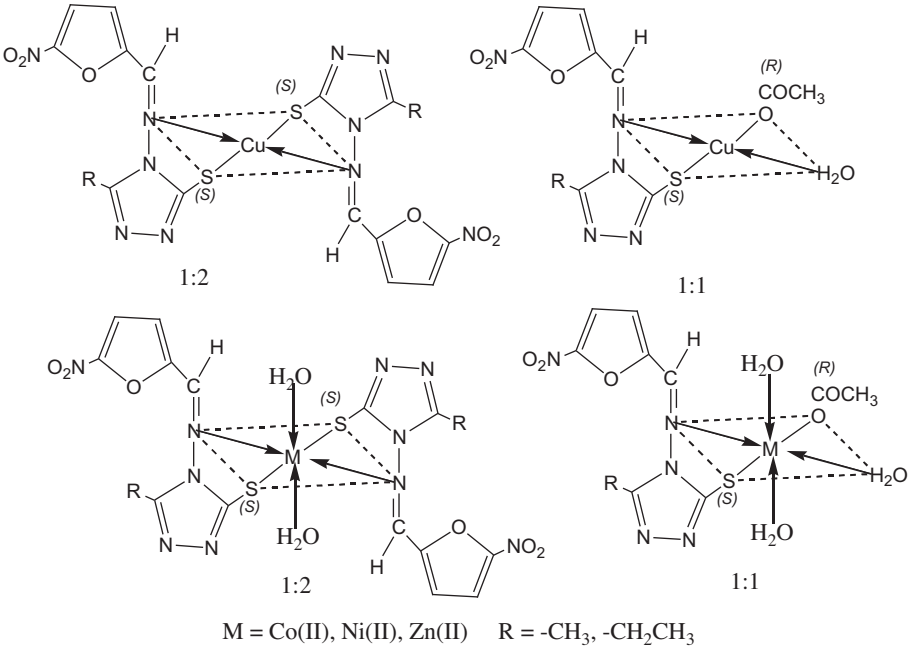


Figure 6 Structure of metal complexes.

of the thiol group. The bonding of ligand to metal ion is confirmed by elemental analyses, spectral studies (UV–Vis, IR, ^{1}HNMR, Fluorescence), TGA, magnetic and conductance measurements. The proposed structures of metal complexes are presented in Fig. 6. Compounds namely: $Zn(L^1)(OAc).3H_2O$ and $Zn(L^2)(OAc).3H_2O$ were found to exhibit the highest antimicrobial activity and $Zn(L^2)(OAc).3H_2O$ was found to exhibit the highest antifungal property. The antimicrobial studies suggested that the Schiff bases were found to be biologically active and their metal complexes showed significantly enhanced antibacterial and antifungal activities against microbial strains in comparison to the free ligands.

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